A Crystalline Compound of β-Lactoglobulin with Dodecyl Sulfate[2]

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Previous studies[3,4,5,6] have shown that proteins are precipitated from solution by synthetic detergents. Anionic detergents such as dodecyl sulfate precipitate proteins from acid solutions, whereas cationic detergents precipitate proteins from alkaline solutions. In the *p*H region close to the isoelectric point, neither cationic nor anionic detergents form precipitates with proteins.[6] Precipitated protein detergent complexes are soluble in an excess of detergent, accompanied by the denaturation of the protein and the liberation of free sulfhydryl groups.[3] The interaction of proteins with synthetic detergents has been extensively reviewed by Putnam.[7]

The work reported here deals with the preparation and properties of a crystalline complex of β-lactoglobulin combined with small quantities of dodecyl sulfate.

Experimental

Preparation of β-Lactoglobulin.—Crystalline β-lactoglobulin was prepared from unpasteurized milk by the method of Palmer.[8] The β-lactoglobulin contained 15.6% nitrogen and was electrophoretically homogeneous at *p*H 8.4 but inhomogeneous at *p*H 4.7, as was demonstrated by Li.[9]

Purified sodium dodecyl sulfate was used. A 0.01 molar aqueous solution, made to *p*H 4.2 with acetic acid, was used in precipitating β-lactoglobulin.

Preparation of Crystalline β-Lactoglobulin-dodecyl Sulfate.—β-Lactoglobulin–dodecyl sulfate was prepared by several procedures, in which the proportion of dodecyl sulfate to protein ranged from 4.2 to 14.0 cc. of 0.01 molar dodecyl sulfate per gram of protein. In every case, the crystalline protein prepared by dialysis at *p*H 5.1–5.2 after the removal of dodecyl sulfate with barium chloride, as described by Putnam and Neurath,[6] differed from β-lactoglobulin in solubility and mobility. Figure 1 gives a comparison of normal and dodecyl sulfate β-lactoglobulin electrophoretic patterns and mobilities in acetate buffer at *p*H 4.8 and veronal buffer at *p*H 8.4.

By adding 4.4 cc. of 0.01 *M* dodecyl sulfate to 50 cc. of a 2.1% solution of protein at *p*H 4.8, it was possible to crystallize the modified β-lactoglobulin directly, without the preliminary formation of a precipitate or the use of barium chloride to remove dodecyl sulfate. On standing for several hours, characteristic crystals appeared which had the electrophoretic mobility of modified β-lactoglobulin, demonstrating that only a small amount of dodecyl sulfate is necessary to modify the properties of β-lactoglobulin and that barium ions or barium sulfate do not produce the modification. The preparation which has been analyzed most completely was made as follows: approximately 10 g. of crystalline β-lactoglobulin suspended in 250 cc. of water was dissolved in dilute acetic acid and made to *p*H 4.2. Then 70 cc. of 0.1 *N* dodecyl sulfate at *p*H 4.2 was added with stirring. The small amount of precipitate formed was ignored. The solution then was made to *p*H 6.0 by adding dilute ammonia. The excess dodecyl sulfate was precipitated by adding 5 cc. of a 5% solution of barium chloride. After thirty minutes, the precipitated barium dodecyl sulfate was removed by centrifugation, and the supernatant was adjusted to *p*H 5.1. On dialysis, a yield of about 8 g. of crystalline protein was obtained. This material had a mobility u(sq. cm. volt^{-1} sec.$^{-1}$ $\times 10^5$) of $1\ u \times 10^5$ at *p*H 4.7 in acetate buffer and a mobility of $-5.9\ u \times 10^5$ at *p*H 8.4 in veronal buffer of 0.1 ionic strength. After several recrystallizations of the protein by dialysis from salt solutions, the properties were unchanged.

Table I shows the total nitrogen, α-amino nitrogen and total sulfur contents.

Properties of β-Lactoglobulin Dodecyl Sulfate

The analytical data in Table I indicate that two molecules of dodecyl sulfate are bound to one molecule of β-lactoglobulin. The method of preparation involving dialysis and treatment with barium to remove the insoluble barium salt of do-

(1) One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Philadelphia. Article not copyrighted.

(2) A preliminary report of this work was presented at the meeting of the American Society of Biological Chemists, Atlantic City, March, 1948; *Federation Proc.*, **7**, 172 (1948).

(3) Anson, *J. Gen. Physiol.*, **23**, 239 (1939).

(4) McMeekin, *Federation Proc.*, **1**, 125 (1942).

(5) Putnam and Neurath, *J. Biol. Chem.*, **150**, 263 (1943).

(6) Putnam and Neurath, This Journal, **66**, 692 (1944).

(7) Putnam, "Advances in Protein Chemistry," Vol. IV, Academic Press, Inc., New York, N. Y., 1948, p. 79.

(8) Palmer, *J. Biol. Chem.*, **104**, 359 (1934)

(9) Li, This Journal, **68**, 2746 (1946)

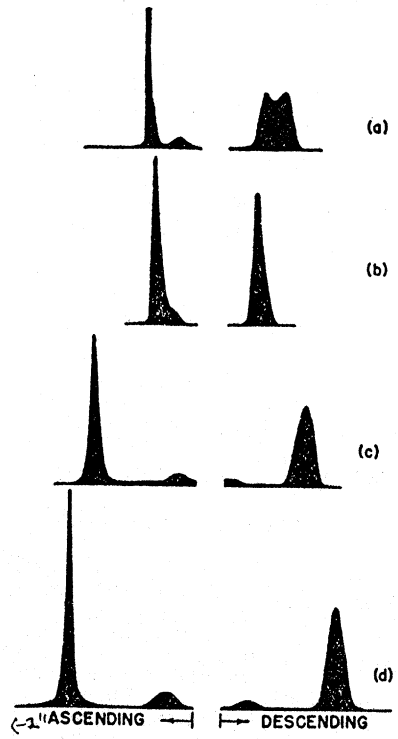

Fig. 1.—Comparison of electrophoretic patterns of normal β-lactoglobulin with those of its dodecyl sulfate derivative. In acetate buffer at *p*H 4.8, ionic strength 0.1: (a) β-lactoglobulin after 10,800 sec. at 4.37 volts/cm., (b) β-lactoglobulin dodecyl sulfate derivative after 10,800 sec. at 4.0 volts/cm. In veronal buffer at *p*H 8.4: (c) β-lactoglobulin after 10,800 sec. at 4.7 volts/cm., (d) β-lactoglobulin dodecyl sulfate derivative after 10,800 sec. at 4.6 volts/cm. Mobilities (a) 1.3, 2.5 *u*, (b) 1.0 *u*, (c) −5.1 *u*, (d) −5.9 *u*.

decyl sulfate indicates that the dodecyl sulfate is firmly held by the protein. It would be of great interest to locate the position of attachment of the dodecyl sulfate to the protein. It was thought that some information on the place of attachment between β-lactoglobulin and dodecyl sulfate might be obtained from a comparison of the properties of the normal and the modified protein.

TABLE I

COMPOSITION OF β-LACTOGLOBULIN AND ITS DODECYL DERIVATIVE

	Weight percentages		
	Total N	α-Amino N	Total S
β-Lactoglobulin	15.67	1.26	1.58
β-Lactoglobulin dodecyl sulfate	15.39	1.24	1.76
Calculated for:			
1 *M* β-lactoglobulin (mol. wt. 35000)			
2 *M* dodecyl sulfate (mol. wt. 265)			
	15.43	1.24	1.74

Titration Curve.—The combination of β-lactoglobulin–dodecyl sulfate with acid and alkali was compared with that of untreated β-lactoglobulin in potassium chloride solutions of 0.1 ionic strength, according to the method described by Cannan, *et al.*[10] Figure 2 shows the results. The combining capacity is consistent with the electrophoretic mobilities, in that at *p*H 4.7 the β-lactoglobulin–dodecyl sulfate with the smaller mobility combines with less acid than does β-lactoglobulin, whereas at *p*H 8.4 the alkali-combining capacity of the β-lactoglobulin–dodecyl sulfate is about two equivalents greater than that of β-lactoglobulin and the mobility is also greater. The two titration curves for solutions more acid than *p*H 4 are essentially the same; apparently the change in dissociation takes place between *p*H 4 and 6, which could not be that of the sulfate group, since it is a very strong acid and does not give a measurable *pK*.

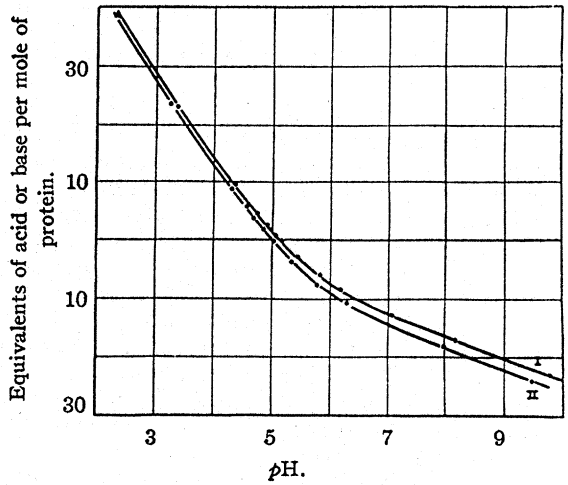

Fig. 2.—Titration curve of β-lactoglobulin (I), mol. wt. 35,000, and of β-lactoglobulin–dodecyl sulfate (II), mol. wt., 35,530.

Solubility.—There is a striking difference in the solubility of β-lactoglobulin and its dodecyl sulfate derivative. With the technique used by Gronwall,[11] the solubility of β-lactoglobulin at 25° was found to be 0.16 mg. of nitrogen per cubic centimeter in water and 1.7 mg. per cubic centimeter in 0.02 *M* sodium chloride. The solubility of dodecyl sulfate derivative was 0.08 mg. of nitrogen per cubic centimeter in water and 0.5 mg. of nitrogen per cubic centimeter in 0.02 *M* sodium chloride. Figure 3 shows the influence of *p*H on the solubility in water in 0.02 *M* sodium chloride.

Temperature of Coagulation.—Luck and his associates[12] have determined the effect of a variety of substances on temperature of coagulation or cloud point of serum albumin. These investigators have reported that small amounts of sodium dodecyl sulfate are particularly effective in preventing urea denaturation of serum albumin.

(10) Cannan, Palmer and Kibrick, *J. Biol. Chem.*, **142**, 803 (1942).
(11) Gronwall, *Compt. rend. trav. lab. Carlsberg*, **24**, No. 8-11, 185 (1942).
(12) Ballou, Boyer, Luck and Lum, *J. Biol. Chem.*, **153**, 589 (1944).

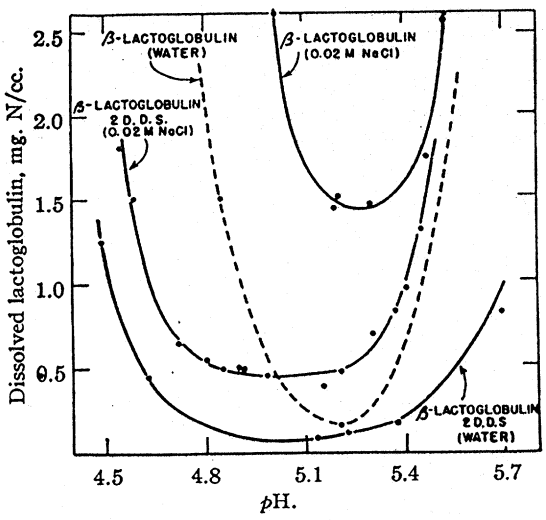

Fig. 3.—Comparison of the solubility of β-lactoglobulin with that of its dodecyl sulfate (D. D. S.) derivative.

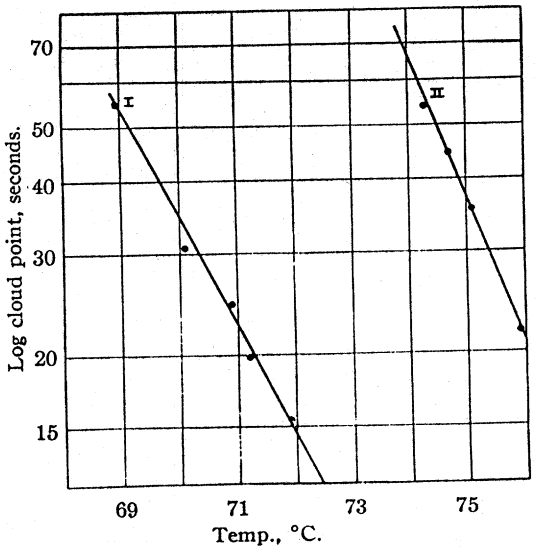

Fig. 4.—Comparison of log cloud point of β-lactoglobulin (I) with that of β-lactoglobulin–dodecyl sulfate (II) (1% solutions in 0.05 *M* sodium chloride, *p*H 5.2).

Cloud points of a 1% solution of β-lactoglobulin and its dodecyl sulfate derivative were determined in 0.05 *M* sodium chloride at *p*H 5.2. These are illustrated in Fig. 4. The presence of combined dodecyl sulfate increases the cloud point of β-lactoglobulin approximately 5°.

Optical Activity.—When dissolved in 0.1 *M* acetate buffer at *p*H 4.8, the β-lactoglobulin-dodecyl sulfate derivative had a specific rotation of $[\alpha]^{25}D$ −23.6°, as compared with a specific rotation of −30.5 for the untreated β-lactoglobulin.

Discussion

Exploratory studies with substances other than dodecyl sulfate, such as the dioctyl ester of sulfosuccinic acid and orange II, indicate that the combination of β-lactoglobulin with small amounts of large anions is rather general. Davis and Dubos[13] have found th t β-lactoglobulin combines with fatty acids, but to a smaller extent than does serum albumin.

Efforts to remove the two equivalents of dodecyl sulfate from β-lactoglobulin have not been successful. Long dialysis at *p*H 8.4 or treatment with barium hydroxide at *p*H 9.0 did not remove the dodecyl sulfate. Modification of the mobility of β-lactoglobulin by the presence of two molecules of unremovable dodecyl sulfate appears to be analogous to the difference in mobility between serum albumin and regenerated detergent-treated serum albumin,[6] indicating that barium chloride does not remove all the detergent from serum albumin.

The electrophoretic results of Li[9] indicate that β-lactoglobulin is heterogeneous. A partial separation of the electrophoretic components has been attained by fractionation,[14] giving crystalline fractions differing in solubility in water and salt solutions. It was hoped that dodecyl sulfate would be a useful substance for separating the components of β-lactoglobulin. However, all the fractions obtained by means of dodecyl sulfate had the same electrophoretic composition and behavior, indicating that the component proteins of β-lactoglobulin were all modified in a similar manner by the addition of dodecyl sulfate.

It is possible that the electrophoretic heterogeneity of β-lactoglobulin is due to the presence of non-amino acid groups combined with a portion of the β-lactoglobulin molecules in a manner similar to the combination of dodecyl sulfate. Electrophoretic determinations made on mixtures of equal parts of β-lactoglobulin and its dodecyl sulfate derivative are consistent with this hypothesis. In veronal buffer at *p*H 8.4, the normal β-lactoglobulin and the dodecyl sulfate derivative have markedly different mobilities, −5.1 and −5.9 *u*, respectively. The mixture moved as a single component with a mobility of −5.7 *u*. At *p*H 4.8 in acetate buffer, the electrophoretic pattern of the normal β-lactoglobulin indicated two components, with 60% of the protein in the faster component. The dodecyl sulfate derivative gave an electrophoretic pattern which was almost homogeneous, being composed largely of a component moving with a mobility of the slowest of the two β-lactoglobulin components. Mixtures of the normal protein and dodecyl derivative had a composite electrophoretic pattern. It will be necessary, however, to await the separation and analysis of the electrophoretic components of β-lactoglobulin before this hypothesis gains further credence.

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(13) Davis and Dubos, *J. Expt. Medicine*, **86**, 215 (1947).

(14) McMeekin, Polis, DellaMonica and Custer, THIS JOURNAL, **70**, 881 (1948).

sulfate; and also to Dr. S. R. Hoover for the α-amino nitrogen determinations and Dr. C. L. Ogg for the sulfur determinations.

Summary

Preparation of a crystalline derivative of β-lactoglobulin containing two equivalents of firmly bound dodecyl sulfate is described. The solubility, mobility, titration curve and denaturation temperatures of the derivative are compared with the corresponding properties of β-lactoglobulin.

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